# The Final Research Report of Memorandum of Understanding (MOU) of No. FA2386-12-1-4065

Title of Proposed Research:

Assembly of Photosynthetic Antenna Protein Complexes from Algae for Development of Nano-biodevice and Its Fuelization

Key Researcher involved in the Proposed Project:

### Mamoru Nango

Affiliation of Researcher:

Nagoya Institute of Technology

Address of Researcher:

Advanced Institution of Monotsukuri, Nagoya Institute of Technology Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan

<Duration of the proposed project> From May 24, 2012 To May 23, 2013

Report Documentation Page				Form Approved OMB No. 0704-0188	
maintaining the data needed, and coincluding suggestions for reducing	ompleting and reviewing the collect this burden, to Washington Headqu ald be aware that notwithstanding an	o average 1 hour per response, inclu- ion of information. Send comments arters Services, Directorate for Infor ny other provision of law, no person	regarding this burden estimate mation Operations and Reports	or any other aspect of the 1215 Jefferson Davis	is collection of information, Highway, Suite 1204, Arlington
1. REPORT DATE 10 JUN 2013		2. REPORT TYPE		3. DATES COVE	RED
4. TITLE AND SUBTITLE  Assembly of Photosynthetic Protein Complexes from Algae for  Development of Nanobiodevices and Their Fuelization				5a. CONTRACT NUMBER	
			e for	5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NU	JMBER
Mamoru Nango				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan, NA, NA				8. PERFORMING ORGANIZATION REPORT NUMBER	
			goya	N/A	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
				11. SPONSOR/M NUMBER(S)	ONITOR'S REPORT
12. DISTRIBUTION/AVAIL Approved for public		ion unlimited.			
13. SUPPLEMENTARY NO	TES				
control the direction nanobiodevices (na algae as well as from throughout the nealight-harvesting (La light harvester of analogous to photometrical control of the control	n and orientation on nobiophotonics) and plants and photonics of UV to near IR regels of the well-established synthetic antenna for the manual properties.	photosynthetic anter f the complex on ele d its fuelization. The synthetic bacteria is gion and much high from these photosy d cell in order to cor rom algae for develo- ntion?s systems of hy	ctrodes with patt e advantage of the its high efficience er durability usin enthetic membran estruct an efficience opment of new ty	ern for devel ese pigment of y of light-ene g these methous. Our goal at energy tran pe of nanobio	opment of complexes from orgy conversion ods than ordinary is to use the LH as asfer system
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF	18. NUMBER	19a. NAME OF
a. REPORT	b. ABSTRACT	c. THIS PAGE	ABSTRACT	OF PAGES	RESPONSIBLE PERSON

unclassified

unclassified

unclassified

### Contents

Ι.	Abstract of the project results	3 р			
Π.	I . Result of the project $4~\mathrm{K}$				
	1. Introduction	4 p			
	2. Experiment	8p			
	3. Results & Discussion	8 р			
	4. List of Publications	15p			
	A. Journal paper publications B. Conference presentation	15p 16p			

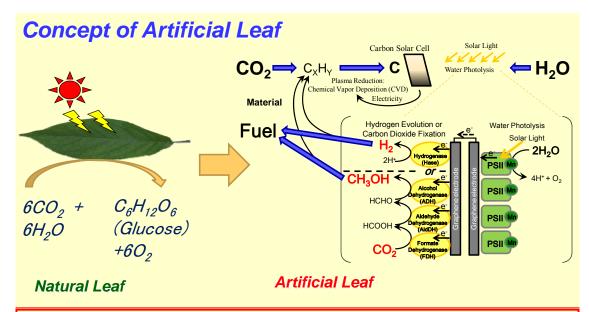
### I. Abstract of the project results

### **Abstract**

The purpose of this proposal is to use photosynthetic antenna pigment complex (LH) from algae in order to control the direction and orientation of the complex on electrodes with pattern for development of nanobiodevices (nanobiophotonics) and its fuelization. The advantage of these pigment complexes from algae as well as from plants and photosynthetic bacteria is its high efficiency of light-energy conversion throughout the near UV to near IR region and much higher durability using these methods than ordinary light-harvesting (LH) complex isolated from these photosynthetic membranes. Our goal is to use the LH—as a light harvester of the well-established cell in order to construct an efficient energy transfer system analogous to photosynthetic antenna from algae for development of new type of nanobiodevices and nano-baiomaterial ("artificial leaf" as shown in Scheme 1), constructing fuelization's systems of hydrogen and carbon dioxide.

The present generations of sensor and semiconductor devices with an integrated circuit for developing nano-level size are too expensive to be cost-effective compared to other existing technologies. Expanding existing silicon device technologies and nanofabrication technics by incorporation of modified photosynthetic protein / pigments complexes or their protein-mimic materials to perform tasks of light-harvesting and charge separation, is currently explored as a novel concept, which makes use of natural protein environments to create a directional flow of light energy and electronic charge separation, meanwhile reducing the cost aspect by the use of bio-materials and their synthetic protein-mimic materials. The majority of the aim is construction of the array of artificial photosynthetic antenna system with nano-patterning substrate using modified photosynthetic protein materials prepared from modern biosynthetic manufacturing methods and photosynthetic pigments.

We proposed a scenario where the construction of electron transfer system analogous to artificial photosynthetic system from algae with patterning substrate is expected to start from molecular and supra-molecular entities in a variety of smart matrices that collect light energy and separate charge for developing new types of nano-biodevices and for the artificial leaf to construct the fuelization system.



Photosynthesis and redox proteins are well-organized into thylakoid membrane in natural leaf, in which glucose and oxygen produce from  ${\rm CO_2}$  and water with assisted visible light energy as shown in this scheme.

Concept: natural leaf is the device for solar fuel production from CO<sub>2</sub> and water.

On going: Development of artificial leaf for solar fuel hydrogen or methanol production from CO<sub>2</sub> based on the photosensitizer, electron carrier (artificial co-enzyme )immobilized onto substrate devices.

### Scheme 1.

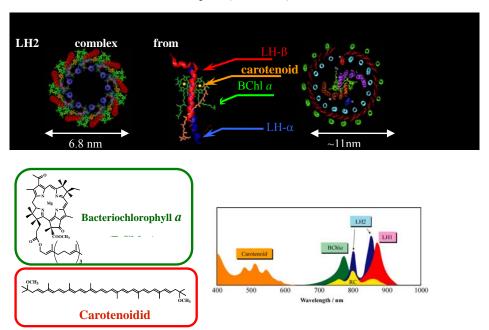
### II. Result of the project.

### 1. Introduction

Nature provides a number of examples, in which processes of energy conversion, storage and transport are combined and optimized through 'smart matrices' at various levels, going from molecular to cellular or higher organisms. Based on biological design principles, future biology-based photonics or their synthetic organic materials could form clean and inexpensive future alternatives for productions of nano-semiconductors and constructing fuelization's system of hydrogen and carbon dioxide (the **artificial leaf**).

The past 10 years have seen tremendous progress in our understanding of the structure and function of the pigment-protein complexes involved in the primary reactions of bacterial photosynthesis. The structure of the reaction center (RC, the first membrane protein to have its structure determined to high resolution) revealed the nearly C<sub>2</sub> symmetrical arrangement of the redox centers and this system has now been extensively studied by ultrafast laser spectroscopy. More recently the structures of the LH2 complexes has revealed the nonameric and octameric arrangement of repeating units consisting of two apoproteins and one or two carotenoids and three BChls while the recent crystal structure of the LH1-RC core complex reveals that the LH1 complex surrounds the contours of the RC although a high-resolution structure has not

yet been determined for the LH1 complex (Scheme 2).



Scheme 2. Compartimentalization of light -harvesting and charge separation.

The antenna complexes(LH2,LH1-RC) efficiently realize various photosynthetic functions using cofactors (BChl a and carotenoid) assembled into the apoproteins (LH1 and LH2).

The light-harvesting mechanisms in these light-harvesting complexes have been studied both spectroscopically and theoretically. These advances put us in a unique position of being able to exploit this information to design artificial photosynthetic antenna systems based on 'biological blueprint'. Our aim is to see if we could produce an antenna module, which acts as a 'sensitizer', and a light-induced redox component for nano-biophotonics and nano-biomaterial (the **artificial leaf**) for fuelization. As well as using LH2 and LH1-RC from photosynthetic bacteria (Scheme 2) this summary also propose to use photosynthetic pigment complex from algae and plants (Scheme 3) or their model complexes as a light harvester of the well-established cell. One of its unique features is that it works over a large dynamic range of incident light intensities. It has a remarkable ability to capture efficiently photons even at very low light fluxes, yet at the same time to withstand very high light fluxes by efficiently dissipating the excess photons. Thereby, protecting itself against the potential harmful effects of over-excitation.

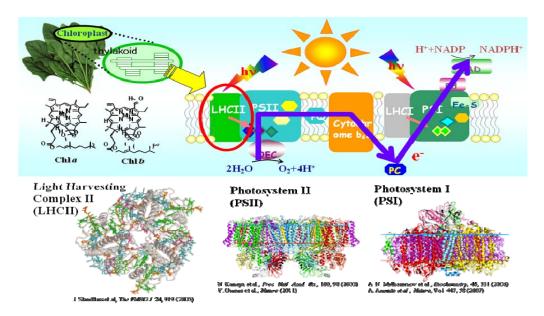
It is important to understand not only the mechanisms of efficient light-harvesting but also those of photo-protection. In order to understand these reactions both structural and functional information is required. The data on how the energy levels and intermolecular interactions of the pigments affect their energy-transfer properties, and how the 'durability' of the complexes is required for rational design of novel biophotonics. Based on the experiments using the native photosynthetic antenna complexes, a variety of modified complexes will be synthesized and tested for their usefulness in artificial nano-biophotonics and its fuelization. After elucidation of the mechanisms of harvesting, transferring, usage and dissipation of light energy, our aim—is to optimize under a given light—intensity the energy-conversion efficiency and

the durability of the core and the antenna complexes by modifying the pigment Cars and BChls or chlorophylls as well as the supporting peptides. These modified photosynthetic protein-mimic complexes was introduced into a membrane system on electrodes with pattern as a light-induced redox component, and the antenna complexes was attached to electrodes modified with or without lipid bilayers as a UV and Vis light harvester modules to produce a new type of nanobiophotonics and nano-biomaterials for fuelization. These approaches provided a foundation for using the artificial domains of photosynthetic core-antenna and antenna complexes , isolated fron algae or plants with patterning substrate and the development of new type of nano-biodevices and nano-biomaterials ( the **artificial leaf**) for fuelization.

### **Approach**

The present generations of sensor and semiconductor devices with an integrated circuit for developing nano-level size are too expensive to be cost-effective compared to other existing technologies. Hence there is a need for productions of sensors and semiconductors using novel-low-cost, systems with the inherently high photon-capturing and charge separation efficiency of natural photosynthetic systems. Integration of photosynthetic proteins or it pigments complexes with nano-patterned devices for tasks of light-harvesting and charge separation will expand existing silicon device technologies and nano-fabrication technics using novel and inexpensive bio-components. Design principles of natural photosynthetic units will form the guideposts for the design and development of native light-harvesting and photoconversion matrix modules as described in the section of a plan of work bellow. A critical step is creating functional supra-molecular nano-assembly of small organic building blocks that co-operate to create a directional flow of energy and electron using the operational principles of the natural systems. Properties of the building-block molecules intrinsically have the capacities to direct their co-operative assembly into structures with specific orientation and alignment. The advantages of the large scale of modern biosynthetic manufacturing methods offers a promising route to economically viable devices.

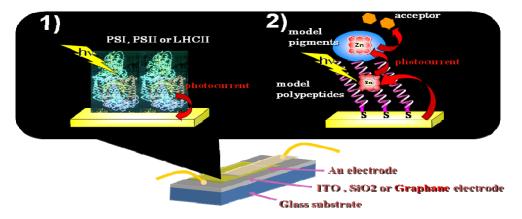
Our goal is to use photosynthetic pigment complex, FCP from algae, LHCII from plants, and PSII and PSI from algae or plants (Scheme 3 for plants) as well as LH2 and LH1-RC from photosynthetic bacteria (Scheme 2) or their model complexes as a light harvester of the well-established cell to convert light energy in the ultraviolet and visible region into that in the near infrared region for development of new type of nano-sensors and nano-biomaterials (artificial leaf). The advantage of the light-harvesting complex is its efficient capture of photons throughout the near UV to near IR region and much higher durability than ordinary isolated dyes supported by its inherent photo-protective function. Thus, the results of the above grounds can be directly applied to the development of nano-photosensors and nano-biomaterials using modified photosynthetic pigments or their model light-harvesting materials with nano-patterning substrate.



**Scheme 3.** Compartimentalization of light harvesting (LHCII) and charge separation (PSII &PSI). The antenna complexes (LHCII, PSII and PSI) efficiently realize various photosynthetic functions using cofactors (Chl *a* and carotenoids) assembled into the apoproteins.

In the current of our previous study, LH1 polypeptides with cysteine group or His-tag at the C- or N-terminal, analogous to the native LH polypeptide from photosynthetic bacteria has been assembled on Au or ITO electrode. In this study, pigments such as native and chlorophyll or carotenoid derivatives were further selected and assembled on the specific site of these polypeptides to control the organization of PSI PSII and LHCII (Scheme 4) which were more stable than the LH-RC or RC complex from photosynthetic bacteria and their model complexes on electrodes modified with or without lipid bilayers. The structural effects of the pigments and the polypeptides on the production of the efficient electron flows were examined.

Further, molecular assembly of porphyrin and carotenoid model pigments on electrodes using synthetic hydrophobic model polypeptide which have similar amino acid sequences to the hydrophobic core in the native photosynthetic antenna light-harvesting polypeptides, PSI, PSII, FCP and LHCII (Scheme 4) was achieved. This method was useful for the self-assembly of these complexes in order to study the energy transfer and electron transfer reactions (capture of photons) between individual pigments in the supra-molecular complexes on the electrodes with pattern for developing nano-biodevices and nano-biomaterials.



**Scheme 4.** Artificial domains of photosynthetic pigment complex on various electrodes: Schematic model of the assembly of PSI, PSII and LHCII and their model complexes on an electrode

### 2. Experiment

More details are presented in our papers in the list of publication and in the abstracts of some representative papers attached.

### 3. Results and Discussion

Molecular self-assembly of photosynthetic pigment complex as shown in Schemes 2 & 3 and their model complexes onto various electrodes was used to develop new types of antenna-mimics nano-sensors. In the current of our previous study, we used modified photosynthetic antenna complex with His-tag or modifiers at the LH polypeptide with SH using molecular biological methods to control the orientation and direction of the complex onto electrodes as shown in Scheme 4.

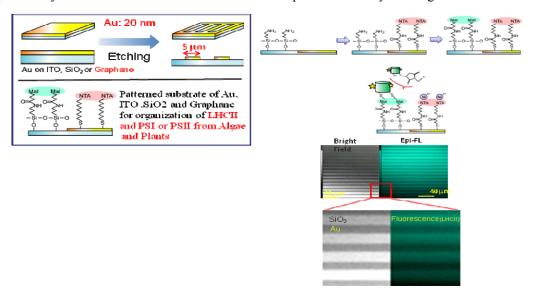
1. Artificial domain assembly of LH pigment complexes from photosynthetic bacterial membranes on nano-patterning and lipid modified substrates to construct efficient energy harvesting and electron transfer systems.

Task 1: Artificial domain assembly of LH pigment complexes from photosynthetic bacterial membranes on nano-patterning and lipid modified substrates to construct efficient energy harvesting and electron transfer systems (A.Sumino, M. Nango, et.al., *Biomacromolecules*, 12, 2850-2858 (2011) & M. Kondo, M. Nango, et.al., *Biomacromolecules*, 13, 432–438 (2012))

The pigment-protein complexes of the modified photosynthetic pigment complex was laid down onto functionalized electrodes, such as ITO, Au and SiO2 electrodes modified with or without lipid bilayers. Upon illumination photocurrents were successfully measured. Excitation spectra confirmed that these photocurrents was produced by light absorbed by the pigment-protein complexes as shown in our previous data (M. Ogawa, M. Nango, et.al., *Chem. Lett.*, 772-773 (2004) and M. Kondo, M. Nango, *Biomacromolecules*, **8**, 2457-2463 (2007), A.Sumino M. Nango, et.al., *Langmuir*, **27**, 1092-11099 (2011)) .

It proved critical in these studies to capitalize on our knowledge of the behavior of these complexes to select those that are the most stable and well organized. The best results was only obtained with the subset of the most stable complexes, the combination of LHCII and PS II-Histag on Au electrode as well as the combination of LH2-SH and C-Histag LH1-RC assembly onto SiO2 and Au, respectively which the orientation and direction of these complexes are controlled. These studies was examined to correlate the supramolecular organization of the complexes on the electrodes with an efficient capture of photons.

AFM and EM studies resolved the organization of antenna complexes both in reconstituted lipid bilayers and in native photosynthetic membranes. These techniques are now being applied to investigate the organization of the antenna complexes and their synthetic model complexes on the electrodes. This work required very careful attention to detail and the current pictures are very exciting.



**Scheme 5.** Schematic model of the assembly of photosynthetic pigment complex (LHCII,PSII and PSI) with pigments on various substrates with pattern.

A clear fluorescence of LH2 with SH-tag was observed at the Mal sites on the substrate with lined patterning when illuminated at near IR region(see Scheme 5) in our previous AOARD report. Following this study, LH1-RC with His-tag was further assembled on the NTA site to produce an efficient energy transfer from LH2 to LH1-RC on the same substrate for development of new type of nano-sensors and nano-semiconductors (nanobiophotonics). We could see an enhanced photocurrent of LH1-RC due to the co-assembly of LH2 on the same electrode with this patterning (To be submitted.). This method of approach will be useful for constructing a new type of solar cell.

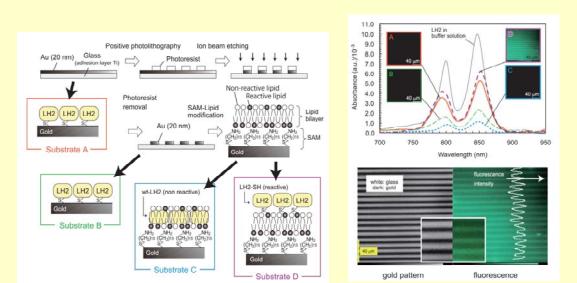
On-going: LH2 with SH-tag and LH1-RC with His-tag will be co-assembled on the Au electrode with more fined pattern to produced enhanced energy transfer systems (see, Task 2.).

## Task 2: Assembly of LH2 with SH-tag on a patterned gold electrode to construct an efficient energy harvesting system.

Following the Scheme 5 in the Task 1, clear fluorescence lines can be observed on gold electrode with more fined pattern due to the presence of lipid bilayers (substrate D). In contrast, no fluorescence was observed without lipid bilayers.

This result fist showed that a method for yielding the fluorescence of LH2 complex was demonstrated by using a lipid-modified surface on the gold substrate, which is more easy for patterning in comparison to other substrates (see: Scheme 6) (Ref. 1: Apply Phys. Lett., 100,233701 (2012), Ref. 7. J. Phys. Chem. B, in press).

This method of approach, applying to gold surface is very useful for constructing a new type of solar cell with more fined pattern.



The first slide(Left): In substrates A and B, the thiol C-terminus of LH2-SH is directly bonded to the gold surface. In substrates, C and D, the gold surface is modified with SAM assisted lipid bilayer. In substrate C, wt-LH2 is incorporated into the lipid bilayer, whereas in substrate D LH2-SH is chemically bonded with a reactive lipid on top of the lipid bilayer. The second slide(Right): (top) Absorption spectra and fluorescence images of substrates A-D. (bottom Transmissionimage (left) and fluorescence image (right) of the same region of substrate D using 765-855 nm bandpass filters. Clear fluorescence lines can be observed due to the presence of lipid bilayers (substrate D). In contrast, no fluorescence was observed without lipid bilayers. A method for yielding the fluorescence of LH2 complex was first demonstrated by using a lipid-modified surface on the gold.

This method of approach, applying to gold surface is very useful for constructing a new type of solar cell with more

This method of approach, applying to gold surface is very useful for constructing a new type of solar cell with more fine pattern. On going: LH1-RC with His-tag will be further co-assembled on a Au electrode with more fined pattern to produce an enhanced energy transfer system.

### Scheme 6.

### Task 3: Artificial domain assembly of LH1-RC on lipid modified substrates to construct an efficient energy transfer system.

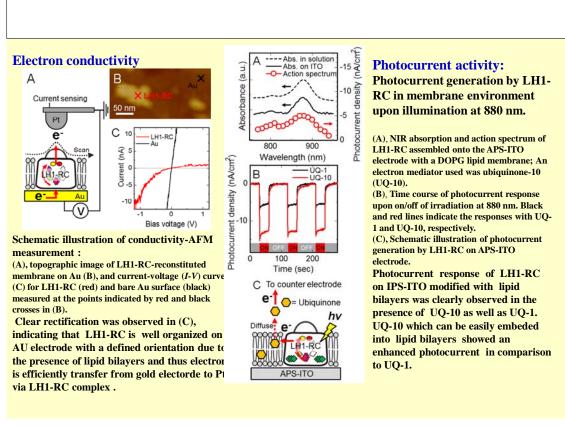
From measurement of the conductive AFM, clear rectification was observed in (C), indicating that LH1-RC is well organized on AU electrode with a defined orientation due to the presence of lipid bilayers and thus electron is efficiently transfer from gold electrode to

Pt via LH1-RC complex (Ref.8: submitted.).

Further, from result of the photocurrent measurements, photocurrent response of LH1-RC on IPS-ITO modified with lipid bilayers was clearly observed in the presence of UQ-10 as well as UQ-1 as shown in Scheme 7.

UQ-10 which can be easily embedded into lipid bilayers showed an enhanced photocurrent in comparison to UQ-1(see: Scheme7)(Ref.6: *J. Phys. Chem. Lett.*, 4, 1087–1092(2013)).

This method of approach using lipid bilayers will be very useful for the assembly of the LH complexes on electrodes with pattern and a defined orientation for construction of solar cell with the functions of photo-response and photoelectric currents.



Scheme 7.

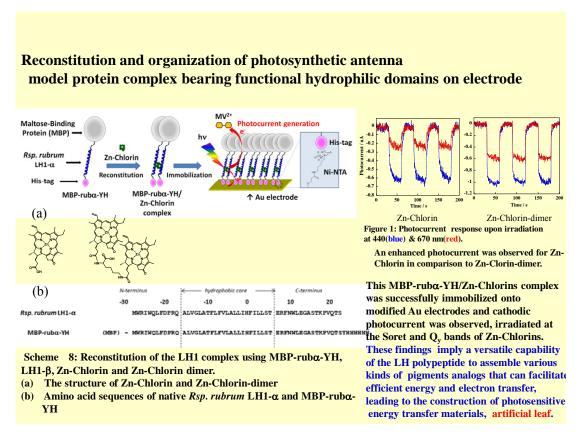
2. Artificial domain assembly of LH pigment complexes and their model complexes to construct energy transfer systems and artificial leaf for the fuelization.

## Task 1: Artificial domain assembly of LH pigment complexes and their model complexes to construct energy transfer systems.

This MBP-ruba-YH/Zn-Chlorins complex was successfully immobilized onto modified Au electrodes and cathodic photocurrent was observed, irradiated at the Soret and Q<sub>y</sub> bands of Zn-Chlorins as shown in Scheme 8 (Ref.5: *Langmuir*;

### 29,5104-5109(2013)).

These findings imply a versatile capability of the LH polypeptide to assemble various kinds of pigments analogs that can facilitate efficient energy and electron transfer, leading to the construction of photosensitive energy transfer materials, artificial leaf as well as solar cell.



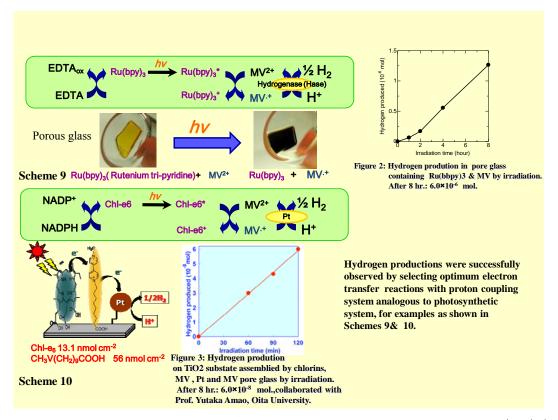
### Scheme 8.

Taken together, the results of this study suggest that the genetically engineered MBP-rub $\alpha$ -YH is useful for construction of artificial photosynthetic antenna systems based on the promising methodology using functional hydrophilic domains, His-tag and MBP for immobilization onto electrodes with a defined orientation and as a molecular landmark for AFM observation at the molecular level, respectively (Ref. No. 5, *Langmuir*, 29,5104-5109(2013)).

### Task 2: Artificial domain assembly of LH pigment complexes and their model complexes to construct an artificial leaf for hydrogen production.

Hydrogen production was successfully observed in porous glass by selecting optimum electron transfer reactions with proton coupling system, for an example as shown in Scheme 9. Further, hydrogen production was successfully observed on an electrode by selecting optimum

electron transfer reactions with proton coupling system, for an example as shown in Scheme 10.



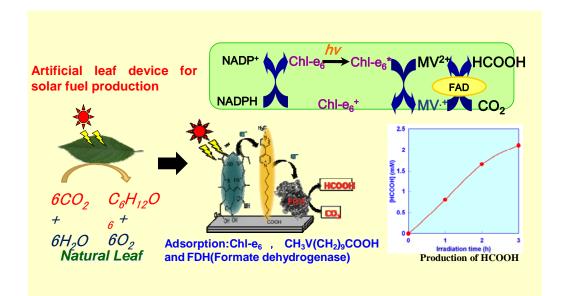
These schemes 9 & 10 show the assembly of redox enzyme model, Ru(bpy)<sub>3</sub>( Rutenium tri-pyridine)+ MV<sup>2+</sup>) in pore glass or Chl derivative, Chl-e6 + MV on the electrode with hydrogenase or Pt analogous **the LH complexes system** to produce hydrogen for developing artificial leaf of fuelization (see the scheme 1).

This method of approach using enzyme model assembled in pore glass and on electrode with pattern will be very useful for construction of artificial leaf with the functions of solar to fuel, hydrogen production.

On-going, we will use LHCII, FCP, PSI and PSII as redox enzyme in near future as the concept for an artificial leaf (see, Schem 1).

### Task 3: Artificial domain assembly of LH pigment complexes and their model complexes to construct an artificial leaf for fuelization of carbon dioxide

Fuelization from carbon dioxide to formic acid was successfully observed by selecting optimum electron transfer reactions with proton coupling system as shown in Scheme 11, analogous to photosynthetic system, collaborated with Prof. Yutaka Amao, Oita University (Bull. Chem. Soc. Jpn, 2009, 82, 93-95)



Scheme 11. Artificial domain assembly of LH pigment complexes and their model complexes to construct an artificial leaf for fuelization of carbon dioxide

On going: LHCII, FCP, PSI and PSII with FDH, AldDH, and ADH coupled with proton transfer will be used for further assemblies in pore glass and on electrodes to fuelization of carbon dioxide to methanol production (the artificial leaf as shown in Scheme 1).

Summary: The present generations of sensor and semiconductor devices with an integrated circuit for developing nano-level size are too expensive to be cost-effective compared to other existing technologies. Expanding existing silicon device technologies and nanofabrication technics by incorporation of modified photosynthetic protein / pigments complexes such as PSI, PSII, FCP and LHCII from algae or their protein-mimic materials to perform tasks of light-harvesting and charge separation, is currently explored as a novel concept, which makes use of natural protein environments to create a directional flow of light energy and electronic charge separation, meanwhile reducing the cost aspect by the use of bio-materials and their synthetic protein-mimic materials. Based on biological design principles, future biology-based photonics or their synthetic organic photonics could form clean and inexpensive future alternatives for productions of nano-sensors and nano-semiconductors.

In this report, we proposed a scenario for the construction of artificial photosynthetic system ( the artificial leaf ) with patterning substrate, starting from molecular and supramolecular entities in a variety of smart matrices to lead an electrochemical potential for development of new types nanobiodevices and fuelizing carbon dioxide.

### Pay-off

### Effects of dissemination of research results are as follows,

- 1) Assembly of photosynthetic antennas and their protein-mimic complexes on electrodes with nanopattern. This proposal aims to incorporate modified core complex (PSII and PSII) and the antenna (LHCII) complexes, and their model complexes onto Au, ITO and SiO2 with pattern. If this trial becomes successful, it can trigger the development of a new information technology, IT industry as well as development of a new new type of nano-photonic sensors and nano-semiconductors..
- 2) Efficient usage of light energy. Photosynthetic antennas can collect light energy in the entire region from ultraviolet to near infrared. It has a unique property to harvest a small number of photons from all the different directions and to concentrate them for usage. This mechanism to enable high sensitivity in a wide spectral region can be used as a guiding principle in designing the artificial leaf as shown in the Scheme 1 as well as photo-electronic materials.
- 3) Key to solve the energy and environmental crisis. Development of a safe and economical system for conversion of light energy into electricity is crucial in order to solve the energy and environmental crisis. The photosynthetic system is a best refined material in harmony with the global environment, and the present project aims to create "Solar to Fuel" as well a novel battery or sensors for the next generation using the solar energy which is exhaustible, clear and free of pollutant.

#### 3. List of Publications:

### A. Journal Paper publication:

- 1. S. Yajima, R.A.: Furukawa, M. Nagata, S. Sakai, M.Kondo, K. Iida, T. Dewa, M. Nango, "Two-dimensional patterning of bacterial light-harvesting 2 complexes on lipid-modified gold surface", *Apply Phys. Lett.*, **100**,233701 (2012).
- 2. J. Nakamura, M. Maekawa, M. Nango, "Peroxide decoloration of azo dyes catalyzed by manganese chlorophyll derivative in micellar solutions", *Textile Res. J.*, **83**,1396-1401(2012)
- 3. M. Yamamoto, T. Horibe, Y. Nishisaka, S. Suzuki, M. Kozaki, R. Fujii, M. Doe, M. Nango, K. Okada, H. Hashimoto, "Reassociation of All-trans-3,4-Dihydroanhydrorhodovibrin with LH1 Subunits Isolated from Rhodospirillum rubrum: Selective Binding of All-trans Isomer from Mixture of cis- and trans-Isomers", *Bull.Chem.Soc. Jpn.*, **86**,121-128 (2012)
- 4. T. Dewa, A. Sumino, N. Watanabe, T. Noji, M. Nango "Energy Transfer and Clustering of Photosynthetic Light-Harvesting Complexes in Reconstituted Lipid Membranes" *Chem. Phys.* in press.
- 5. S. Sakai, T. Noji, M. Kondo, T. Mizuno, T. Dewa, T. Ochiai, Y. Hisanori, S.Ito, H.Hashimoto, M. Nango, "Molecular Assembly of Zinc Chlorophyll Derivatives by Using Recombinant Light-Harvesting Polypeptides with His-tag and Immobilization on a Gold Electrode", *Langmuir*, 29,5104-5109(2013)(dx.doiorg/10.10201/la400059hl).
- 6. A.Sumino, T. Dewa, N. Sasaki, M. Kondo. M. Nango, "Electron Conduction and Photocurrent Generation of Light-Harvesting/ Reaction Center Core Complex in Lipid Membrane Environments", *J. Phys. Chem. Lett.* 4, 1087–1092(2013), (dx.doi.org/10.1021/jz301976z).
- 7. S.Tubasum, S.Sakai, T.Dewa, V.Sundstrom, S. Ivan, M.Nango, Mamoru, T. Pullerits, "Anchored LH2 Complexes in 2D Polarization Imaging", *J. Phys. Chem.B*, in press.

- 8. A. Sumino, T. Dewa, T. Noji, Y.Nakano, N.Watanabe, R. Hildner, N. Bösch,
- J. Köhler, M. Nango, "Phospholipids Modulate Self-Assembled Nanostructure and Energy Transfer of the Light-Harvesting Complex 2 in Lipid Bilayers", submitted.

### B. Conference presentations (International conference):

1. International Conference of AFM, "Seeing at the Nanoscale" July 10, 2012, Bristol, UK.

#### **Abstracts**

### AFM observation of supramolecular assembly of light-harvesting membrane proteins in lipid bilayers/supramolecular structure and their function

Ayumi SUMINO, Takehisa DEWA, Natsuko WATANABE, YukiNAKANO,
Tomoyasu NOJI, and Mamoru NANGO
(Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-kuNagoya466-8555,
Japan )

**Key Word:**photosynthetic antenna proteins / supramolecular assembly / atomic force microscopy / energy transfer

[Introduction] In a bacterial photosynthesis, light-harvesting complex 2 (LH2) and lightharvesting-reaction center complex (LH1-RC) play the key roles of capturing andtransferring light energy and subsequent charge separation. The photosynthetic apparatus form molecular assembly, however, how the assembly influences the efficiency of the energy conversion is not yet clear. To address this issue, we reconstituted LH2 and LH1-RC into lipid bilayer and evaluated the structure of LH2 and LH1-RCby atomic force microscopy (AFM) and the intermolecular fluorescence resonance energy transfer from LH2 to LH1-RC.

**[Experiment]** LH1-RC and LH2 isolated from purple photosynthetic bacteria, were reconstituted into liposomal membrane. A suspension of lipid/detergent/membrane protein was dialyzed against detergent-free buffer, giving LH2- or LH1-RC-reconstituted liposomal (proteoliposome) solution.

[Results and Discussion] Figure 1 shows AFM images of LH2 (A) and LH1-RC (B) incorporated into lipid bilayers. The LH2 molecules whose structures are cylindrical (d=6 nm) can be clearly observed (Figure 1A).

AFM image of LH1-RC clearly shows protruded part (H-subunit of RC, h = -3 nm) surrounded by ellipsoidal structure (LH1, d = -13 nm) (Figure 1B). These observed structures were in good agreement with corresponding crystallographic structures. [1, 2] For functional analysis of these reconstituted complexes, we observed the intermolecular fluorescence resonance energy transfer from LH2 to LH1-RC in micellar and liposomal solutions in Figure 2A and 2B, respectively. The molar ratio of these complexes was LH2/LH1-RC = 1/1 in each solutions. In the case of the LH2-only membrane (black line) fluorescence from B850 was observed upon irradiation at  $\lambda_{ex} = 800$  nm (B800 of LH2). The LH1-RC-only membrane (gray line) showed very weak fluorescence upon irradiation at 800 nm. (Emission from LH1-RC appears at 900 nm upon excitation of B880 band.) When the LH1-RC was added into the LH2 micellar solution (spectrum colored in blue in Figure 2A), fluorescence from LH2 ( $\lambda_{em} = 867$  nm) was slightly suppressed and band was broadened. This indicates that energy transfer from LH2 to LH1-RC takes place in the micellar solution. In sharp contrast, when both LH2 and LH1-RC complexes were incorporated into a liposomal membrane (spectrum colored in blue in Figure 2B), fluorescence from LH2  $(\lambda_{em} = 867 \text{ nm})$  was significantly suppressed and that from LH1-RC

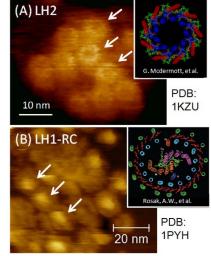


Figure 1. AFM images of LH2 (A) and LH1-RC (B) in lipid bilayers.

 $(\lambda_{em} = 895 \text{ nm})$  was prominently enhanced. This clearly shows enhanced energy transfer from LH2 to LH1-RC in the reconstituted membranes. [3]

- [1] G. McDermott, et al., Nature 1995, 374, 517–521.
- [2] A. W. Rosak, et al., Science 2003, 302, 1969–1972.
- [3] A. Sumino, et al., e-J. Surf.Sci. Nanotech.2011, 9, 15-20.

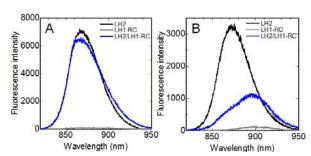


Figure 2.Fluorescence spectra of LH2 and/or LH1-RC in micellar (A) and liposomal (B) solutions. Black line, LH2; gray line,LH1-RC;blue line,LH2/LH1-RCcoexisting system.